Emergence Success and Sex Ratio of Commercial Alfalfa Leafcutting Bees from the United States and Canada

THERESA L. PITTS-SINGER AND ROSALIND R. JAMES

USDA–ARS Bee Biology & Systematics Laboratory, Utah State University, Logan, UT 84322

J. Econ. Entomol. 98(6): 1785–1790 (2005)

ABSTRACT Samples of overwintering alfalfa leafcutting bee, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), cells were sent to the laboratory as loose cells or in nesting boards from bee managers in the United States and in Canada. X-radiographs of cells were used for determining cell contents. Cells containing live prepupae were incubated, and the sex of emerging adults was recorded daily. Cells from which no adult emerged were dissected to determine the developmental stage of dead bees and sex of dead pupae or adults. Bee cells incubated in commercial settings and placed in alfalfa fields by the same bee managers described above also were evaluated to determine adult emergence success. The proportion of live bees in wood nesting boards from the United States was much lower than the live proportion in polystyrene nesting boards from Canada and loose cells overwintered in the United States. For laboratory-incubated loose cells, survival and sex ratios of bees from Canadian sources were statistically higher than those of U.S. bees, but the onset and duration of emergence times were similar. Fewer bees survived in the commercial setting than in the laboratory. Prepupal mortality was significantly higher than pupal or adult mortality, but there was no significant difference between the sexes in the likelihood of survival during incubation. This study supports the commonly held belief that alfalfa leafcutting bees raised in Canada and then sold to the United States represent a more viable source of bees than most bees produced in the United States.

KEY WORDS Apiformes, Apoidea, Megachilidae, *Medicago*, lucerne

ALFALFA LEAFCUTTING BEE, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), is a cavity-nesting bee that has been used as a commercial pollinator of alfalfa, *Medicago sativa* L., since the 1960s (Stephen 1955; Bohart 1957, 1972; Stephen and Torchio 1961; Stephen 1962, Hobbs 1964, 1967, 1972; Richards 1984). In commercial operations, nesting sites are provided for the bees in the form of holes in wood or polystyrene boards. These boards are mounted vertically in domiciles (shelters) that are placed in alfalfa fields during the summer growing season. Each female bee creates nest cells, one at a time, by lining the nesting tunnel with pieces of leaves she cuts from local alfalfa plants. The female bee provisions the cell with pollen and nectar and then lays an egg on top of the provision mass; thus, each cell has the potential to produce one adult bee. The sex of the bee is determined by whether the egg is fertilized. Female bees are produced from fertilized eggs laid preferentially in the first few nest cells in a cavity (Gerber and Klostermeyer 1970, Maki and Moffett 1986, Jay and Mohr 1987, O'Neill 2004). Males are produced from unfertilized eggs laid in cells nearest the cavity opening. The bees overwinter in the cells as diapausing prepupae and emerge as adults in the spring or early summer depending upon incubation conditions. The slightly smaller and faster developing males emerge before females. Although males are important for fertilization, female bees are more desirable than male bees for commercial purposes because females are the more efficient pollinators of alfalfa flowers (Cane 2002).

A system called loose cell management commonly used in the commercial management of the alfalfa leafcutting bee helps to reduce the incidence of chalkbrood disease, parasites and predators, and cells with unused provision, while also reducing space needed for winter storage and shipping (Bohart 1972, Richards 1984, Baird and Bitner 1991). In this system, bee cells are removed by punching them from polystyrene or wood boards or by stripping them from stacked, laminated (grooved), polystyrene or wood pieces (Baird and Bitner 1991). The loose cells are then usually tumbled in a screened drum so that cells containing chalkbrood-infected larval cadavers break apart and fall out of the tumbling apparatus, along with insect predators, other broken cells, and leaf material (Baird and Bitner 1991, Frank 2003). Cells that do not contain prepupae break apart because they lack the silken cocoon that holds together the cell's leaf pieces (Peterson et al. 1992). Other processes that managers may use are mechanical, air, and/or gravity methods for cell separation (unpublished data). Inadvertent loss of some cells occurs from mechanical damage that kills otherwise viable prepupae.

The loose bee cells, as well as bee-filled nesting boards, are stored for several months over the winter. Approximately 2.5–3 wk before predicted peak bloom in alfalfa fields, bees are incubated at ≈30°C to initiate the final stages of development to pupae and then adults (Stephen and Osgood 1965, Richards 1984, Peterson et al. 1992, Frank 2003). Under such conditions, emergence of males is expected to begin on day 18–20 of incubation, and female emergence follows 2 to 3 d later (Richards 1984, Frank 2003). Emergence of all adults ends within 1 wk after it starts (Peterson et al. 1994). If inclement weather creates a risk of mortality to adults released in the field, or delays the onset of the alfalfa bloom, the incubation temperature can be reduced to 15–20°C to slow adult emergence (Rank and Goerzen 1982, Richards 1984, Stephen and Fichter 1992).

In 1990, U.S. alfalfa seed growers spent nearly $11 million to purchase alfalfa leafcutting bees for pollinating seed alfalfa (Peterson et al. 1992). Because the market for contract alfalfa seed fluctuates, and because the price of leafcutting bees changes with relative supply and demand, seed growers must evaluate the economic viability of obtaining enough bees to pollinate the crop. To meet their pollination needs, most alfalfa seed growers in the northwestern United States must purchase all or a proportion of their bees from suppliers in Canada. Some U.S. growers have the equipment and space to raise their own bees, but are unable to produce sufficient numbers of viable bees. The first step in addressing the problems of alfalfa leafcutting bee production in the United States is to compare commercial bee populations produced in the United States and Canada, and for this study we examined the incubation phase of bee management. Our objective was to determine adult sex ratio, successful emergence, and synchronization of emergence in relation to the sources of bees and management techniques during the incubation period.

Materials and Methods

In spring 2003 and 2004, samples of alfalfa leafcutting bee cells were solicited from different bee managers. Some managers were alfalfa seed growers who raise their own bees, and others were entrepreneurs (i.e., pollinator specialists) who manage bees for pollination services on seed growers' farms. Therefore, the bee cells we obtained were from several northwestern states of the United States and from southwestern Canada. Some Canadian bees were shipped directly from Canada, whereas others were shipped from U.S. alfalfa seed growers who had recently purchased Canadian bees. There were 22 samples of bees from Canada, although the bee producers were not revealed. Samples of bees produced in the United States were from Idaho ($n = 5$), Oregon ($n = 3$), Montana ($n = 5$), Utah ($n = 3$), Washington ($n = 11$), and Wyoming ($n = 4$). Bees were shipped to the laboratory on or around the day upon which the bee manager initiated the process of incubation at his own facility. Most samples were loose cells, although there were five samples received as sections of nest boards containing bee cells.

For each sample of bee cells that arrived at the laboratory, at least 500 random cells were X-radiographed (Stephen and Undurraga 1976) and scored according to whether they contained healthy prepupae, dead bees, or other contents (i.e., parasites, pests, or unused provision). Those cells containing live prepupae were removed, transferred to petri dishes (15 by 2.5 cm), and incubated in the dark at 29°C. Incubating only the cells containing live prepupae eliminated the possibility of early emerging hymenopteran parasitoids affecting adult emergence. Approximately 2 wk after the start of incubation, emergence of adult bees was evaluated daily. Once adult bee emergence began, petri dishes were checked at 0700–0900 hours and again at 1600–1800 hours, and the sex and number of emerged bees were recorded. At least 1 wk after the last adult bee had emerged from an individual sample, the remaining, intact cells were dissected to determine the number and developmental stages of bees that had failed to emerge. When possible, the sex of dead pupae and adults also was determined.

Alfalfa leafcutting bee cells also were obtained from field incubation trays or nest boards of commercial bee managers after adult bee emergence in alfalfa seed fields had been completed. At least 500 cells from each sample were evaluated to determine the number that were empty because of adult bee emergence and the number of intact, nonviable cells. Intact cells were dissected as described above to determine their contents.

For the loose cells, logistic analysis of variance (ANOVA) (SAS Institute 1999) was used to determine whether the country of origin (United States or Canada) had a significant effect on the proportion of cells containing live prepupae, dead bees (adult, prepupae, and pupae), or other contents. For live prepupae incubated only in the laboratory, the onset and duration of adult (male and female) emergence for each laboratory sample was determined and the adult sex ratio (m:f) was calculated. A logistic ANOVA was used to test whether the country of origin and year the sample was taken had any effect on the duration of incubation onset to the emergence of the first males and females, and on the total duration of emergence for each sex. Logistic ANOVA also was used to determine whether the country of origin significantly affected the sex ratio. To determine whether proportionately more bees died as prepupae, pupae, or unemerged adults, logistic ANOVA was performed using country of origin and bee incubation location (laboratory or commercial setting) as main effects.

Results

For all samples of loose, overwintered bee cells, some cells contained dead bees before incubation (Fig. 1). The percentage of live prepupae in the U.S. samples ranged from 9.8 to 85.2%. The highest proportions of live prepupae were obtained from two of the Montana samples and one of the Wyoming sam-

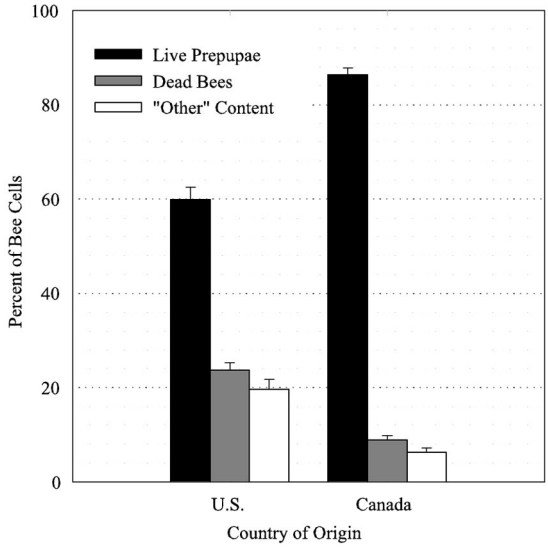

Fig. 1. Mean percentage and standard error of *M. rotundata* cells obtained from the United States and Canada containing live prepupae (black bars), dead bees (gray bars), and other contents (white bars). Data are from X-ray analyses of bees before incubation in the laboratory.

ples. The lowest proportions of live prepupae were found in three of the samples originating from Oregon. The percentage of live prepupae in the Canadian samples ranged from 63.2 to 94.8%. There was a significantly higher proportion of live prepupae (χ^2 = 2129.25, df = 1, $P \leq 0.0001$), lower proportion of dead bees (χ^2 = 871.34, df = 1, $P \leq 0.0001$), and lower proportion of cells containing other contents (χ^2 = 890.55, df = 1, $P \leq 0.0001$) in the Canadian samples (Fig. 1). In the U.S. samples, fewer of the bees stored in nesting boards were alive than in the loose cell system, but the two storage systems had nearly the same survival in the Canadian samples. Polystyrene boards containing overwintered bees from Canada ($n = 2$) contained high percentages of live prepupae (91.7 and 86.1%) compared with wood boards managed similarly in the U.S. ($n = 2$; live prepupae, 1.1 and 47.6%). The third U.S. wood board sample was of a board that had been used for renesting for two consecutive years, and the live count in this board was <0.2%.

In the laboratory, under uniform incubation conditions for all samples, we found that the average onset of male emergence was about 1 d before the first emergence of any female (Table 1). There was no significant difference between countries of origin for either sex in the number of days to first emergence or the duration of emergence or in the number of days from the beginning of incubation until all the surviving bees had emerged (Table 1). The sex ratio of emerged adults from Canadian sources was significantly more male-biased than the sex ratio of adults from U.S. sources (χ^2 = 206.96, df = 1, $P \leq 0.0001$) (Table 1).

In all board samples, the bees died more often as prepupae than as pupae or adults during incubation. Survival to the adult stage was high for both of the Canadian board samples (90.2 and 86.1%). One U.S. board sample yielded 91.6% successful emergence of the bee cells deemed healthy before incubation, but no adults emerged from the two U.S. board samples that had very few live prepupae before incubation. Duration of emergence (average 30.5 d) and sex ratio (average 2.45) from the two Canadian polystyrene board samples were slightly higher than that from the Canadian loose cell samples. From the U.S. wood board sample, the emergence duration was lower (25 d), and the sex ratio higher (1.71) than those obtained from the U.S. loose cell samples (Table 1).

For the loose cells studied, a significantly higher proportion of live adults emerged from Canadian samples than from U.S. samples (χ^2 = 318.6, df = 1, $P \leq$ 0.0001). Additionally, significantly more bees survived to adult emergence when they were incubated in the laboratory than when they were incubated by the commercial manager (χ^2 = 756.6, df = 1, $P \leq 0.0001$) (Table 2). The interaction term for these two factors was also significant (χ^2 = 14.1, df = 1, $P \leq 0.0002$) because the negative impact of commercial incubation conditions was slightly greater for U.S. bees than for Canadian bees (Table 2). Mortality of males and females (as pupae or adults) did not differ significantly (χ^2 = 2.3, df = 1, $P \leq 0.134$).

Most of the bees that failed to survive the incubation period died as prepupae (Table 2). On average (based on the log odds ratios), prepupae were 4.7 times more likely to be found dead than pupae and 8.3 times more likely to be found dead than adults. Pupae were 1.8 times more likely to have died than adults. Mortality was always lowest for the adult stage and highest for the prepupal stage; the differences between the three life stages were always significant (based on the 95% CI for the odds ratios). However, the degree of difference was significantly affected by country of origin

Table 1. For incubation of *M. rotundata* adults, mean (and standard error) number of days until the first emergence of males and females, the duration of emergence for each sex, and the total number of days from the start of incubation until the end of emergence in the laboratory

Country of origin (n)	Male emergence (d)		Female emergence (d)		All adults (M:F) (d)	
	Until first emergence	Duration	Until first emergence	Duration	From incubation to emergence	Sex ratio*
United States (32)	17.44 (0.29)	8.78 (0.47)	18.69 (0.30)	9.34 (0.58)	27.50 (0.66)	1.26 (0.07)
Canada (22)	17.27 (0.38)	10.05 (0.64)	18.59 (0.40)	9.41 (0.59)	27.59 (0.69)	1.91 (0.15)

Sex ratio of emerged adults is number of males per number of females.
* Significantly different between countries of origin at $P \leq 0.0001$.

Table 2. Average percentage (and standard error) of live and dead life stages of *M. rotundata* prepupal cells (deemed live before incubation) that were incubated under laboratory conditions (Lab) and percentage (and standard error) of live and dead life stages of only viable *M. rotundata* cells (e.g., cells not parasitized or with unused provisions) from commercial conditions (Comm)

Country of origin (*n*)	Live adults		Dead adults		Dead pupae		Dead prepupae	
	Lab	Comm	Lab	Comm	Lab	Comm	Lab	Comm
U. S. (10)	86.6 (1.8)	71.8 (2.5)	0.7 (0.1)	2.2 (0.4)	1.3 (0.2)	3.1 (0.5)	8.3 (1.3)	14.5 (1.5)
Canada (6)	93.1 (1.0)	80.8 (3.1)	0.3 (0.1)	1.2 (0.3)	0.5 (0.1)	3.0 (0.5)	4.7 (0.7)	7.7 (1.3)

All parameters showed a significant effect of country of origin and location of incubation at $P \leq 0.0001$.

($\chi^2 = 210.0$, df = 1, $P \leq 0.0001$) and whether the bees were incubated in the laboratory or in a commercial setting ($\chi^2 = 673.1$, df = 1, $P \leq 0.0001$, for all interactions) (Table 2). Furthermore, all the interaction terms were highly significant ($\chi^2 \leq 34$, df ≤ 2, $P \leq 0.0001$), an indication that the degree of difference was also dependent on the origin of the bees and how they were incubated.

Discussion

For the alfalfa seed production industry, it is important that overwintering alfalfa leafcutting bee prepupae survive the winter, develop to adulthood during incubation, and live long enough to pollinate fields of alfalfa flowers. A self-sustaining system for alfalfa seed production requires that bees flown in the summer produce enough healthy progeny to replace or increase the parental population. From this study, it was apparent that the bees produced in Canada (whether overwintered as loose cells or in nesting boards) were more likely to survive wintering and incubation periods than those produced by most U.S. bee managers. Some of the bees sampled that had been raised and wintered in the United States were first generation descendants of bees purchased from Canada the previous field season, yet these bees still did not survive as well as the bees raised in Canada. The exceptions to this generality are the few bee managers from Montana and Wyoming whose proportions of viable bees were nearly as high as those from Canada. The proximity of these states to the alfalfa-growing regions of Canada is an indication that the environment and length of the nesting season may be underlying factors in sustaining healthy bee populations. However, no definitive reason for the discrepancy in success between regions has been confirmed, and many possible causes exist.

Certain problems in alfalfa leafcutting bee production are incurred during the nesting season, such as chalkbrood disease and early mortality. In Canada, the use of paraformaldehyde during incubation gives the Canadian alfalfa leafcutting bee producers an effective control for the spread of chalkbrood disease in bee brood, whereas this product is not registered for such use in the United States (Goerzen and Watts 1991, Goettel et al. 1993, Goettel and Duke 1996, Frank 2003). Although chalkbrood disease may cause bee losses as high as 65% (James 2005), it is only one mortality factor found in alfalfa leafcutting bees during the nesting season. Early brood death and broodless provisions are also very important (Pitts-Singer 2004), and more effort is needed to determine what management, environmental, and genetic factors contribute to the differences in immature bee loss found between alfalfa leafcutting bee populations raised in the United States and Canada.

Differences between regions during management of other phases of this bee's life cycle also may contribute to variability in survival. In this examination of bee survival after overwintering, the average laboratory emergence rates obtained using a 29°C incubation temperature began ≈3 d sooner than Richards (1984) reported for incubation at 30°C (17 versus 20 d for males; 19 versus 22 d for females). The average duration of male emergence in the U.S. samples reported here is similar to emergence reported by Richards (1984), but male emergence in the Canadian samples took 1 d longer. The average number of days from the beginning of incubation until the completion of emergence for bees from both countries was about 3 d shorter than that reported by Richards (27 versus 30 d). The data from this study fall between two of many incubation temperature regimes examined by Rank and Goerzen (1982) in which bee cells were incubated at 30°C from days 1–14, at 15°C (regime 1) or 30°C (regime 2) for days 15–19, and the following days up through emergence at 30°C. Using these temperature regimes, the mean incubation durations were 29 and 24 d, respectively. Discrepancies between our results and those of others undoubtedly is influenced by differences in bee population sources and exact incubation temperature regimes, but also may be due to variations in overwintering storage temperatures and duration of prewintering and wintering periods (Krunic and Hinks 1972). At least 7 mo of winter storage at 5°C is recommended for a high rate of survival and rapid completion of adult emergence after incubation at 30°C (Richards et al. 1987).

To ensure that pollination occurs early in the production season, predictable and shorter emergence periods are desired for synchronization of bee release with alfalfa bloom. Warm weather occurs earlier and lasts longer in most alfalfa regions in the United States than in Canada, which might lead to local adaptations in development and emergence times for bees from those regions. However, we found that U.S. bees did not begin to emerge any sooner than Canadian bees and that U.S. emergence durations were only slightly shorter than those of Canadian bees. Shorter emergence durations for the U.S. bees simply may have been because fewer adults survived to emerge.

M. rotundata females are the primary pollinators in alfalfa, thus the bee managers and seed growers need to know the sex ratio of overwintering prepupae to determine how many bees to incubate. The operational sex ratio is usually estimated to be two males to each female (Peterson et al. 1992). This study showed that the average sex ratio of bee populations from Canada was closer to this expected value than the more female-biased bees surveyed from the United States, which was closer to one male per female. Maternal determination of offspring sex can be affected by variations in nest tunnel diameter and nesting medium (Stephen and Osgood 1965, Gerber and Klostermeyer 1972), tunnel length (Gerber and Klostermeyer 1972, Mayer 1994), intertunnel distance (Tepedino et al. 1994), and the weight of individual mass provisions (Klostermeyer et al. 1973). From this study, it is not possible to conclude that any of the aforementioned factors contributed to the different sex ratios found, although many of the collaborators in this study used similar nesting materials. However, more dead prepupae were found in the U.S. samples than in the Canadian samples before incubation. The sex of these prepupae is unknown but could have been biased toward males, explaining at least some of the sex ratio differences in surviving adults between the two regions. For the dead pupae and adults, where sex was determined, we found that neither sex was more likely to die than the other during incubation, under either laboratory or commercial conditions.

Regardless of the country of origin or where bee cells were incubated, bees did die during the incubation process. Dead prepupae, pupae, and adults represented a loss of ≈12–15% of the potential adult bees. Although we found no difference in mortality between males and females as pupae or adults, Richards and Whitfield (1988) found in their Canadian bees that more male than female pupae died during incubation at 25–35°C. In this study and that of Richards and Whitfield (1988), mortality occurred primarily in the prepupal stage. Prepupal mortality implies that the bees were unable to continue their maturation, and we propose that prepupae may have experienced suboptimal conditions during prewintering or wintering periods (Johansen and Eves 1973, Kemp and Bosch 2000). Suboptimal temperature regimes or the imposition of inappropriate development and diapause periods may inhibit prepupae from metamorphosing to the next life stage. Improper management during critical life stages might lead to such problems as a lack of energy storage, such that bees die in the spring even if they live through the winter (Bosch and Kemp 2000, 2003; Buckner et al. 2004). Suboptimal conditions also may affect one sex more than the other. Why conditions may be suboptimal for bees of only one sex or from only some sources remains to be determined.

Acknowledgments

We greatly appreciate the technical assistance provided by Ellen Klomps and Peggy Reiger (USDA–ARS, Logan, UT) and by undergraduate assistants Jace Beattie, Elizabeth Sharp, and Marti Jo Watterson (Utah State University, Logan, UT). We thank James Cane (USDA–ARS, Logan, UT), Wayne Goerzen (SASPA/SASPDC, Canada), and Ruth O'Neill (Montana State University, Bozeman, MT) for valuable reviews of this manuscript. We are grateful to the many bee managers from the United States and Canada who generously cooperated with us.

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Received 16 May 2005; accepted 20 July 2005.